Harris Lines: A Study of Age-Associated Bias in Counting and Interpretation

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ABSTRACT Harris lines are regularly used in paleopathology as indicators of episodic nonspecific stress, but the methodology for their use has not been clearly established. We studied radiographs of the distal shaft of the tibia in 82 immature and 49 mature subjects from a medieval burial site and compared the number of Harris lines and observer error according to age categories. We found statistically significant differences in both line counts and in observer error by age groups. In conclusion, studies of Harris lines must take into account age variation in order to be validated. Am J Phys Anthropol 103:209–217, 1997. © 1997 Wiley-Liss, Inc.

Harris lines (HL) (Harris, 1926, 1931, 1933) are diaphyseal or metaphyseal transverse lines of increased opacity, visible in x-rays of the long bones of living subjects or skeletal remains. Whatever they are called, whether HL (Wells, 1967), transverse lines (Park and Richter, 1953), lines of increased density (Acheson, 1959; Garn et al., 1968), or opaque transverse lines (Byers, 1991), they have been reproduced in experimental animal studies by fasting, by selective deficiency in essential nutrients, by bacterial inoculation, and by the administration of hydrocortisone acetate (Harris, 1933; Wolbach, 1947; Follis, 1951; Follis and Park, 1952; Acheson, 1959; Platt and Stewart, 1962). It was these experimental studies which linked the formation of HL with the occurrence of a stress.

Histological studies of the mechanism of occurrence of the HL are not recent (Follis and Park, 1952; Acheson, 1959). In the light of present medical knowledge and earlier observations of HL, it is safe to say that an insult is reflected by failure of growth cartilage, affecting both proliferation and cell maturation. If a general stress occurs, it is mediated by the inhibition of the growth hormone–somatomedin axis. The complex structure of matrix substance and mineralisation are most likely modified by this phenomenon because they are entirely dependent upon the action of chondrocytes. Normal balance at the chondrometaphyseal junction and in particular angiogenesis are no doubt disturbed. Osteogenic cells may be transported by the capillaries more deeply into the cartilage, but certainly when they encounter a cartilaginous matrix which is physically and molecularly different, they lay down lamellar bone which differs from normal bone.

In human medicine, the link between the formation of HL and general stress or disease has been demonstrated after calorie

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and protein deficiency (Jones and Dean, 1959; Park, 1964) and vitamin deficiency (Elliot et al., 1927). HL, which are only formed during the period of growth, may be found at all ages, including perinatal subjects after disease in utero (Sontag, 1938) or inadequate maternal nutrition (Steinbock, 1976). Dreizen et al. (1964) found no difference between two groups, one of normal subjects and the other of subjects with obvious signs of stress. They came to the conclusion that the most prominent features in the appearance of HL were the nonspecificity of the cause and the individuality of the response. This study was disputed by McHenry (1968), who emphasized the fact that for HL to appear favorable conditions must again be present, as has been observed by other authors (Greulich and Pyle, 1966; Steinbock, 1976; Buikstra and Konigsberg, 1985; Magennis, 1990). Nevertheless, Dreizen et al.'s observations are disturbing, since it is unlikely that immature subjects seen at an American hospital after World War II would have presented long-lasting pathological states and that conditions adequate for HL formation would not recur. The question of the true difference between Dreizen et al.'s two groups may be raised however, as all the immature subjects were patients at the same hospital.

In any event, it is known that on the one hand, HL appear in healthy immature subjects and if they are linked with stress the existence of infraclinical disorders must be considered, as demonstrated by Zimmerman and Kelley (1982). On the other hand, only 25% of severe diseases in immature subjects result in HL formation (Gindhart, 1969) and this is perhaps due to the discontinuous nature of growth (Oudet and Petrovic, 1977; Hermanussen, 1988; Lampl et al., 1992).

In paleopathology, HL are considered to be an indicator of episodic general stress (Wells, 1961, 1967; McHenry, 1968; Cohen and Armelagos, 1984; Ozonoff, 1988; Cunha, 1995). However the link between the number of HL observed in a bone and the number of stresses that an individual has undergone is not absolute and the studies can only be statistical in a population. Though HL only appear during the period of linear growth of the long bones, that there are peaks in the appearance of these HL, particularly between the ages of 2 and 5 years (Dreizen et al., 1964). Garn and Schwager (1967) also showed that bone remodeling is responsible for the disappearance of a large number of HL, in particular in the adolescent and the mature subject (Hummert and Van Gerven, 1985). Lastly, Macchiarelli et al. (1994) have shown that HL scoring entails remarkable methodological difficulty with a high level of intra- and extraobserver error.

The aim of the present study was to demonstrate that age and the number of HL are related in such a way that any comparison of number can only be done by age categories. In the course of this study, we found that counting HL was particularly difficult. We attempted to find out why this was so, and how to reduce counting errors to a minimum.

MATERIALS AND METHODS

The burial site

The study concerned the medieval burial site of the Place Saint Etienne (St. Stephen's Square) in Toulouse, southern France. From 1986 to 1987, a rescue excavation of the cemetery of the parish of Saint Etienne, which was in use from the early 10th to the early 13th centuries, took place. The excavation yielded 165 immature and 78 mature subjects. The good state of preservation of the skeletons and the high proportion of immature subjects is one of the features of this site.

Radiographical study

We x-rayed the lower half of the diaphysis of the best-preserved tibiae; this was possible in 82 immature subjects (50% of the population) and 49 mature subjects (63% of the population). A 238 $\times$ 298 mm single-layer nonscreen film was used to improve the definition of the trabecular pattern. The tibiae were x-rayed in groups according to size using constants established after successive tests and ranging from 6 milliamps 40 KV to 12 milliamps 44 KV.

Count of HL

Counting was done on a horizontal light box with the aid of a magnifying lens (magni-

fication ×3). The criterion retained was that of Goodman and Clark (1981): "a visible contrast of increased opacity extending across a fourth or more of the tibial shaft." An observer carried out two successive counts of the series of x-rays and then a third count to check only those x-rays where there was a discrepancy between the first two counts. A second observer carried out a further count. We tested various ways of apportioning the HL in sections according to number, to find which divisions would best minimize errors. The divisions used were the following: Division I: no or one HL, two to five HL, six or more HL; Division II: no or one HL, two to four HL, five or more HL; Division III: no HL, one to four HL, five or more HL; Division IV: no HL, one to five HL, six or more HL.

Age

The age of immature subjects was determined according to dental and bone criteria by one of us (S.R.S.) (Murail, 1991). We divided immature subjects into five age categories, expressed in complete years: 0–0.9, 1–4 years, 5–9 years, 10–14 years, and 15–19 years.

The age of mature subjects was assessed by two methods: 1) stages of epiphyseal union of the medial clavicle according to the method of Owings Webb and Suchey (1980), which separated the mature subjects into two groups, those under 30 years and those over 30 years (this method could not be applied in seven mature subjects); and 2) study of the auricular surface according to Lovejoy's method (1985), which consists of eight phases. In order to limit the number of groups, we amalgamated the phases two by two to obtain four groups: 20–30 years, 30–40 years, 40–50 years, >50 years (this method could not be applied in four mature subjects).

Statistical analysis

For analyzing a gross number of HL, a quantitative variable, and age, we used a nonparametric test (Spearman's rank correlation test) because of the small size of the samples. For immature subjects, where the samples were larger, analysis of variance (ANOVA) was also used. For analysis of the number of HL expressed in groups of lines ("division"), a qualitative variable, we constructed contingency tables and took into account the total chi-square. The significance level was fixed at 95%.

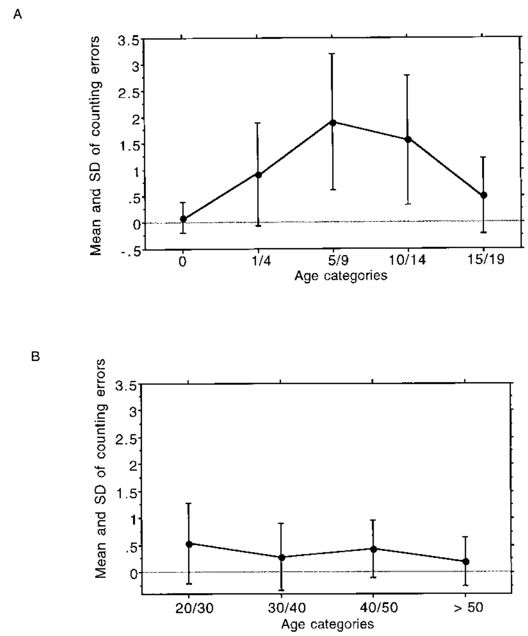


Fig. 1. Correlation between counting errors and age in immature subjects (**A**) and in mature subjects (**B**).

RESULTS

Counting errors

Immature subjects. The mean number of HL during two counts and one check varied little and was always close to 3 (count 1, mean 2.963, SD 2.746; count 2, mean 3.159, SD 2.983; check, mean 3.122, SD 2.895).

On the other hand, when the first two counts were compared, only 50% agreement was found: 28% differed by one HL, 11% by two HL, 8.5% by three HL, and 2.5% by four HL. This made a total of 50% error, of which 11% were serious errors, i.e., the counts differed by three or more HL.

Mean error varied according to age category as shown in Figure 1A. Analysis of variance (Table 1) showed significant differences between the contiguous age categories 0 and 1–4 years, and between 1–4 and 5–9 years. Spearman's rank correlation test, be-

TABLE 1. Counting errors and age[1] in immature and mature subjects

	Counting errors						
Age category	Frequency	Mean	SD	SE	Statistical comparison	Mean difference	Fisher's PLSD
Immature subjects[2]							
<1	22	.091	.294	.063	0 vs. 1–4	−.823	.501*
1–4	35	.914	.981	.166	1–4 vs. 5–9	−.986	.66*
5–9	10	1.9	1.287	.407	5–9 vs. 10–14	.344	.845
10–14	9	1.556	1.236	.412	10–14 vs. 15–19	1.056	1.438
15–19	2	.5	.707	.5			
Mature subjects[3]							
20–30	15	.533	.743	.192	20–30 vs. 30–40	.248	.481
30–40	14	.286	.611	.163	30–40 vs. 40–50	−.143	.599
40–50	7	.429	.535	.202	40–50 vs. >50	.229	.758
>50	5	.2	.447	.2			

[1] Age in years.
[2] ANOVA one factor X1: Age Y1: Error
Spearman rank correlation X1: Age Y1: Error
Z corrected for ties 4.795 $P = .0001$
[3] ANOVA one factor X1: LOVEJOY Y1: Error
Spearman rank correlation X1: Age Y1: Error
Z corrected for ties −.751 $P = .4525$
* 95% significance level.

tween age category and counting error, was significant ($P = 0.0001$).

We tested four different divisions, each with three subdivisions, to attempt to minimize intraobserver error. The frequencies of discrepancy between the first and second counts, expressed for each division, were as follows: division I, 24.39%; division II, 19.51%; division III, 14.63%; division IV, 21.95%. Division III was the one which resulted in the least intraobserver error, which is pertinent since it is made up of a subdivision which is centered on the mean and two subdivisions at −1 and +1 standard deviation. It could be summarized up as follows: no HL, some HL, numerous HL.

The interobserver test gave very similar results to the intraobserver test (43.9% disagreement).

Mature subjects. The mean number of HL during two counts and one check varied little and was always close to 2 (count 1, mean 1.791, SD 2.11; count 2, mean 2.116, SD 2.174; check, mean 1.93, SD 2.098).

Comparing the first two counts, 63% agreement was found: 28% differed by one HL and 9% by two HL. Errors were thus much less frequent in mature than in immature subjects. Mean error was low, ≤0.5%, and decreased slightly with categories of increasing age (Fig. 1B) although the decrease was not significant (Table 1).

Division III (no HL, one to four HL, five or more HL) showed 11.62% disagreement.

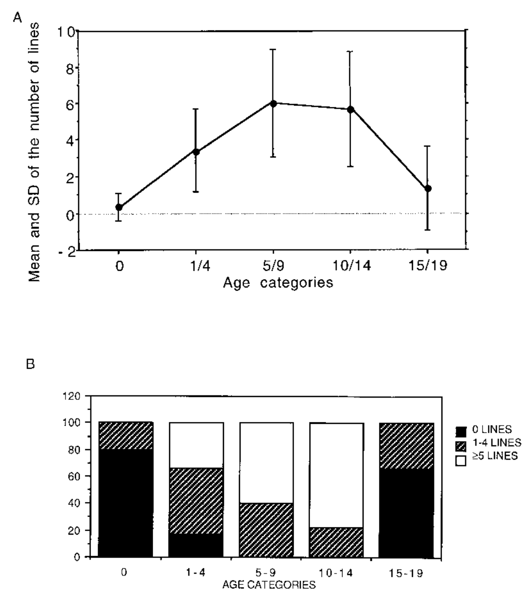


Fig. 2. Correlation between HL and age in immature subjects: calculations based on the gross number of HL (**A**) and on the number of HL expressed according to division III (no HL, one to four HL, five or more HL) (**B**).

Correlation between age and number of HL

Immature subjects. Calculation based on the gross number of HL after checking gave the results shown in Figure 2A. ANOVA (Table 2) showed differences with a significance level of 95% between the following contiguous age categories: 0 vs. 1–4, 1–4 vs.

TABLE 2. Association between Harris lines and age in immature subjects, when the calculations are based on the gross number of Harris lines

Age category[1]	Frequency	Mean	SD	SE	Statistical comparison[2]	Mean difference	Fisher's PLSD
<0	20	.35	.745	.167	0 vs. 1–4	−3.039	1.231*
1–4	36	3.389	2.259	.376	1–4 vs. 5–9	−2.611	1.577*
5–9	10	6	2.981	.943	5–9 vs. 10–14	.333	2.027
10–14	9	5.667	3.122	1.041	10–14 vs. 15–19	4.333	2.942*
15–19	3	1.333	2.309	1.333			

[1] Age in years.
[2] ANOVA one factor X1: Age Y1: LH
Spearman rank correlation X1: Age Y1: LH
Z corrected for ties 5.155 $P = .0001$
* 95% significance level.

TABLE 3. Association between Harris lines and age in immature subjects, when the calculations are based on the number of grouped[1] Harris lines

Harris lines	Age categories in years					
	0	1–4	5–9	10–14	15–19	Totals
0	16	6	0	0	2	24
1–4	4	18	4	2	1	29
≥5	0	12	6	7	0	25
Totals	20	36	10	9	3	78

Values represent the frequency observed.
[1] Division III: 0 HL, 1–4 HL, ≥5 HL. Total $\chi^2 = 44.636$, $P = .0001$.

5–9, and 10–14 vs. 15–19 years, but not between categories 5–9 vs. 10–14 years. Spearman's rank correlation test showed the differences were significant ($P = 0.0001$).

Calculation based on the number of HL expressed according to division III (no HL, one to four HL, five or more HL) gave the results shown in Figure 2B. Statistical analysis was significant, with chi-squared total = 44.636, $P = 0.0001$ (Table 3).

Mature subjects. Calculation based on age as assessed by epiphyseal union of the medial clavicle gave the results shown in Figure 3. The difference between the mean number of HL was significant according to Spearman's rank correlation test (z corrected for ties = −2.702, $P = 0.0069$). However, the difference was not significant if division III was taken into account (no HL, one to four HL, five or more HL) with chi-squared total = 2.1, $P = 0.3499$. This is quite normal given the size of the sample (<30 years [n = 5], >30 [n = 31]) and two degrees of freedom.

Calculations based on age estimated from the auricular surface gave the results shown in Figure 4. A difference nearly reaching significance was obtained by comparing the means of the number of HL using Spearman's rank correlation test, $P = 0.0551$ (Table 4). No significant difference was obtained if we took account of the number of HL in division III (no HL, one to four HL, five or more HL) (Table 5) with chi-squared total = 2.29, $P = 0.8924$, which is normal given the size of the sample (n = 39) and six degrees of freedom.

DISCUSSION

Counting

It was somewhat surprising to see the difficulties encountered when counting HL, due partly to the lack of specificity of Goodman's and Clark's (1981) definition, and resulting eye fatigue. Some counts were very easy, others were particularly difficult. It was evident that the bones of immature subjects of medium height, that is older children and adolescents, are the most difficult to count. This was confirmed by analysis of mean error between two counts, which showed that errors were both most numerous and most serious in the age categories 5–9 and 10–14 years (Fig. 1A and Table 1). In these age groups, HL were numerous, on average about six (Fig. 2A and Table 2) and as bone remodeling was beginning to have an effect, the older HL showed less contrast. By using divisions according to the number of HL, intraobserver error can be kept to a minimum, but multiple counts are essential especially if samples are small. In fact, in this case, the use of divisions according to the number of HL makes statistical analysis difficult by multiplying the degrees of freedom.

Interobserver error was very close to intraobserver error, indicating that its true cause

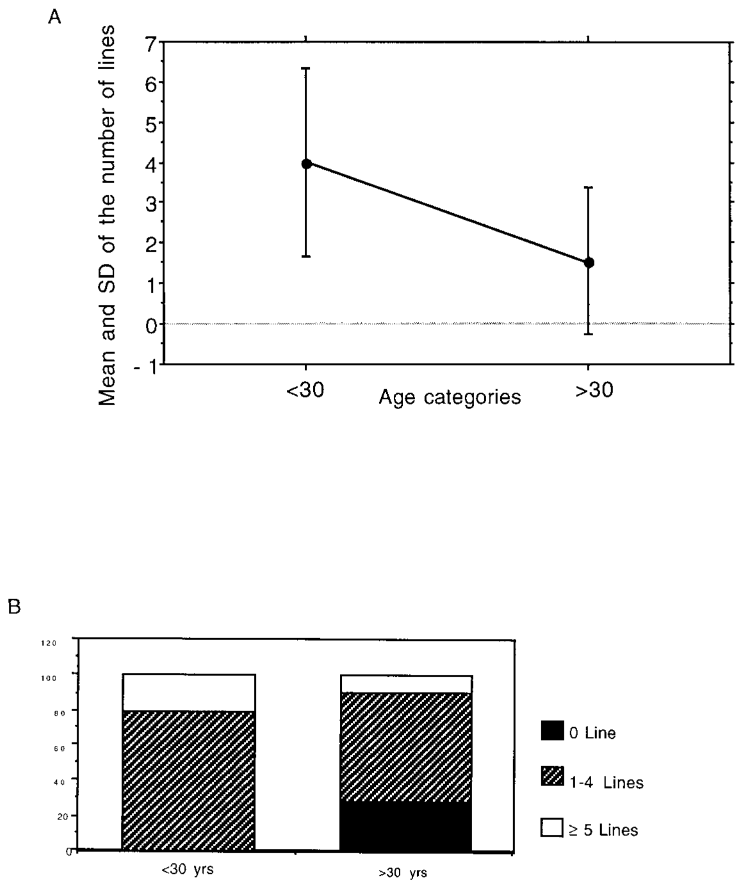


Fig. 3. Correlation between HL and age assessed by epiphyseal union of the medial clavicle in mature subjects: calculations based on the gross number of HL (**A**) and on the number of HL expressed according to Division III (no HL, one to four HL, five or more HL) (**B**).

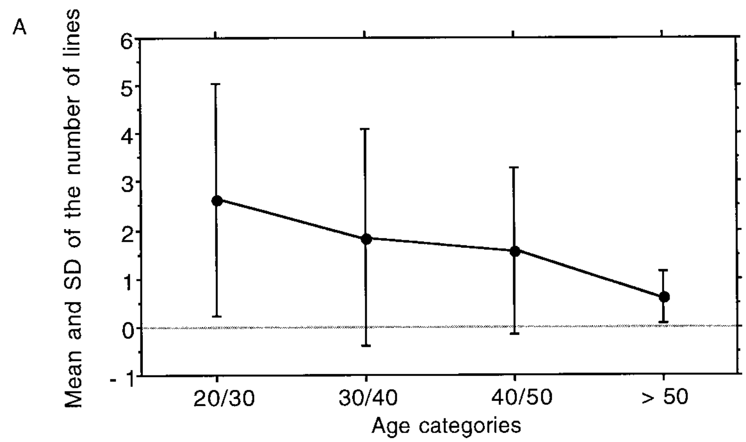
is not an inadequate definition but difficulty of observation. Counting errors were far fewer in mature than in immature subjects and the mean error varied little according to age category (Fig. 1B).

Correlation between age and number of HL

Immature subjects. In these subjects, we found that HL, rare before the age of 1 year, appeared in the 1–4 year age category, became more numerous between the ages of 5 and 9 years, remained stable in the 10–14 age category, and then decreased in the 15–19 age category (Fig. 2A). We observed (Table 3) that no immature subject in the extreme age ranges 0 and 15 years had numerous HL and that the proportion of immature subjects with numerous HL increased in the intermediate age categories (1–4, 5–9, 10–14 years) before decreasing sharply in the 15–19 category (Fig. 2B).

Dreizen et al. (1964) had observed that the formation of HL reached a peak around 3 to 5 years, with a progressive increase between 0 and 5 years and a progressive decrease after the age of 6 years. They considered that this peak corresponded to the conjunction of a high incidence of infectious diseases and high growth velocity. They devoted their attention only to newly formed HL and not to the total number of HL. If we assume that in older children bone remodeling begins to erase HL, then the distribution we found in immature subjects is very similar to that observed by Dreizen et al.

Mature subjects. When age was estimated from the degree of epiphyseal union of the medial clavicle, we observed that



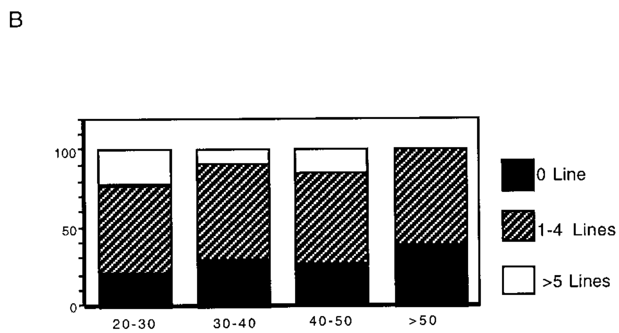


Fig. 4. Correlation between HL and age assessed by auricular surface in mature subjects: calculations based on the gross number of HL (**A**) and on the number of HL expressed according to division III (no HL, one to four HL, five or more HL) (**B**).

TABLE 4. Association of the number of Harris lines and age (assessed by the auricular surface) in mature subjects

Age category	Harris lines: Frequency	Mean	SD	SE
20–30	14	2.643	2.405	.643
30–40	13	1.846	2.23	.619
40–50	7	1.571	1.718	.649
>50	5	.6	.548	.245

Spearman rank correlation X1: Age (Lovejoy) Y1: LH
Z corrected for ties −1.918 $P = .0551$

TABLE 5. Association between Harris lines and age (assessed by the auricular surface) in mature subjects, when the calculations are based on the number of grouped[1] Harris lines

Harris lines	Age categories in years: 20–30	30–40	40–50	>50	Totals
0	3	4	2	2	11
1–4	8	8	4	3	23
≥5	3	1	1	0	5
Totals	14	13	7	5	39

Values represent frequency observed.
[1] Division III: 0 HL, 1–4 HL, ≥5 HL. Total $\chi^2 = 2.261$, $P = .8942$.

young adults (<30 years) presented more HL than older subjects (>30 years) (Fig. 3A). However, the sample of young adults was small (n = 5) and study of HL groups cannot be interpreted even without statistical analysis, since the 20% of young mature subjects (<30 years) with numerous HL only corresponds to a single subject (Fig. 3B).

When age was estimated from the auricular surface (Lovejoy et al., 1985), there was a progressive decrease of the number of HL with increasing age (Fig. 4A and Table 4). Graphic illustration of the number of HL according to age category clearly showed the progressive increase in the number of subjects without HL and the progressive disappearance of those with numerous HL (Fig. 4B and Table 5). Certainly, it could be considered that individuals with numerous HL

had poor health which contributed to an early death, but it is just as legitimate to think that it is not the individuals who have disappeared, but rather the HL, under the influence of bone remodeling.

CONCLUSION

The present work has shown that HL counting is subject to error and that several counts are necessary. Counting is very difficult and is less reliable in immature subjects, in particular between the ages of 5 and 15 years. The use of an appropriate grouping of the number of HL can reduce counting errors to a minimum but requires large samples for statistical analysis to be feasible. Therefore, the number of HL is related to age and any comparative study would need to take into account distribution according to age in order to be validated.

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